

Claims

1. A method for the detection of a target nucleic acid, which method comprises contacting template nucleic acid from a sample with (i) a signalling system and (ii) a tailed nucleic acid primer having a template binding region and the tail comprising a linker and a target binding region, in the presence of appropriate nucleoside triphosphates and an agent for polymerisation thereof, under conditions such that the template binding region of the primer will hybridise to a complementary sequence in the template nucleic acid and be extended to form a primer extension product, separating any such product from the template whereupon 5 the target binding region in the tail of the primer will hybridise to a sequence in the primer extension product corresponding to the target nucleic acid, and wherein any such target 10 specific hybridisation causes a detectable change in the signalling system, such that the presence or absence of the target nucleic acid in the sample is detected by reference to the presence or absence of a detectable change in the signalling system.

15 2. A method as claimed in claim 1 wherein the tailed nucleic acid primer is used as an amplification primer in an amplification system.

3. A method as claimed in claim 2 wherein the amplification system is the polymerase 20 chain reaction (PCR).

4. A method as claimed in claim 2 or claim 3 wherein the tail of the nucleic acid primer remains uncopied during amplification.

25 5. A method as claimed in claim 4 wherein the linker in the tail comprises a blocking moiety to prevent copying of the tail.

6. A method as claimed in claim 4 wherein the tail of the nucleic acid primer comprises a non-copiable species.

7. A method as claimed in any one of the previous claims wherein hybridisation of the tailed primer to template nucleic acid is performed at a stringency so as to allow primer extension on related template sequences.

5 8. A method as claimed in claim 7 wherein the related template sequences are human leukocyte antigen (HLA) sequences.

9. A method as claimed in any one of the previous claims wherein hybridisation of the template binding region and/or target binding region of the primer to a complementary 10 sequence is allele specific.

10. A diagnostic primer for use in a method according to any one of claims 1-9 and comprising (i) a template binding region and (ii) a tail comprising a target binding region and wherein the target binding region hybridises to a complementary sequence in an extension product of the primer corresponding to the target nucleic acid and the complementary sequence is less than 200 base pairs from the template binding region.

11. A diagnostic primer for use in a method according to any one of claims 1-9 and comprising (i) a template binding region and (ii) a tail comprising a linker and a target binding region and wherein the target binding region hybridises to a complementary sequence in an extension product of the primer corresponding to the target nucleic acid.

12. A primer as claimed in claim 10 or claim 11 wherein the template binding region and the tail region are arranged such that the tail region remains uncopied during amplification.

25 13. A primer as claimed in any one of claims 10-12 wherein the linker comprises a blocking moiety which prevents polymerase mediated copying of the primer tail.

30 14. A primer as claimed in any one of claims 10-13 and further comprising at least one component of an integral signalling system to indicate hybridisation of the target binding region to a complementary sequence in an primer extension product of the primer..

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15. A primer as claimed in claim 14 wherein the primer tail carries an intercalating dye.

16. A primer as claimed in claim 14 wherein the primer tail comprises a fluorophore for
5 the detection of target binding by fluorescence polarisation.

17. A diagnostic primer as claimed in claim 14 and further having a separate species
comprising at least one component of an integral signalling system releasably attached to the
primer tail.

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18. A primer as claimed in claim 17 wherein the signalling system comprises energy
transfer between fluorophore and quencher species.

19. A primer as claimed in claim 14 wherein the primer tail acts as a quencher species.

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20. A primer as claimed in claim 13 wherein the primer tail includes one or more regions
of internal hybridisation to stabilise one or more component(s) of the signalling system in a
given position.

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21. A primer as claimed in claim 20 wherein the primer tail comprises a self-
complementary stem duplex having a fluorophore quenched by a quencher species, and
wherein the fluorophore becomes unquenched when the stem duplex is disrupted.

22. A primer as claimed in any one of claims 10-21 which further comprises a capture
25 region which hybridises to complementary sequence on a solid phase.

23. A method as claimed in any one of claims 1-9 and using more than one nucleic acid
primer for the detection of more than one target nucleic acid sequence.

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24. A kit which comprises at least one primer as claimed in any one of claims 10-22
together with packaging and instructions for use.